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Characterization of Na^+ transport across the cell membranes of the ascending thin limb of Henle's loop

NOBUYUKI TAKAHASHI, YOSHIAKI KONDO, IKUMA FUJIWARA, OSAMU ITO, YUTAKA IGARASHI, and KEISHI ABE

Department of Clinical Biology and Hormonal Regulation, Department of Pediatrics, and The Second Department of Internal Medicine, Tohoku University School of Medicine, Sendai, Japan

Characterization of Na^+ transport across the cell membranes of the ascending thin limb of Henle's loop. In the ascending thin limb of Henle's loop (ATL), intracellular Na^+ is extruded by Na^+/K^+ATPase in the basolateral membrane. To further characterize Na^+ transport across the cell membranes of the ATL, the intracellular sodium concentration ($[Na^+]_i$) was monitored using a sodium-sensitive fluorescent probe, SBFI, in the *in vitro* microperfused hamster ATL. Basal $[Na^+]_i$ was 19.0 ± 1.2 mM ($N = 24$). Removal and replacement of luminal Na^+ did not change $[Na^+]_i$ in the presence of Na^+ in the bathing fluid. In contrast, luminal Na^+ removal reduced $[Na^+]_i$ from 11.6 ± 0.9 to 6.3 ± 0.8 mM in the absence of peritubular Na^+ ($P < 0.0005$, $N = 21$). Replacement of luminal Na^+ increased $[Na^+]_i$ to 12.6 ± 0.9 mM. In the absence of Na^+ in the bath, the addition of 1 μM benzamil, 0.1 mM 5-(N,N-dimethyl)-amiloride (DMA), 0.1 mM furosemide, or 0.1 mM trichlormethiazide to the lumen did not change $[Na^+]_i$ or the rate of change in $[Na^+]_i$ ($d[Na^+]_i/dt$) after removal and replacement of luminal Na^+. Decreases in luminal pH in a Hepes-buffered solution and luminal HCO_3^- did not affect $[Na^+]_i$. In the absence of peritubular Na^+, DMA in the bathing fluid decreased $[Na^+]_i$ from 11.4 ± 1.3 to 6.4 ± 1.2 mM ($P < 0.01$, $N = 5$) and completely inhibited the changes in $[Na^+]_i$ after removal and replacement of luminal Na^+. Removal of peritubular Na^+ reduced $[Na^+]_i$ from 18.8 ± 1.2 to 11.3 ± 0.7 mM ($P < 0.0001$, $N = 23$). Addition of DMA in the bathing fluid reduced $[Na^+]_i$ and inhibited the changes in $[Na^+]_i$ after removal and replacement of peritubular Na^+. Addition of benzamil, furosemide or trichlormethiazide to the bathing fluid did not alter $[Na^+]_i$ or the changes in $[Na^+]_i$ after removal and replacement of peritubular Na^+. Decreases in peritubular pH in a Hepes-buffered solution and peritubular HCO_3^- did not affect $[Na^+]_i$. These results indicate that Na^+ transport across the cell membranes of the ATL is mediated by the Na^+/H^+ antiporter in the basolateral membrane together with Na^+/K^+ATPase in the basolateral membrane and demonstrate the absence of Na^+ permeability in the luminal membrane of the ATL.

The ascending thin limb (ATL) of Henle's loop plays an important role in the formation of concentrated urine by the countercurrent multiplier system. It has long been debated whether active Na^+ absorption occurs in the ATL [1–4]. Gottshalk and Mylle [5] provided the first direct evidence for very high permeability of Na^+ in the hamster ATL. In 1965, Marsh and Solomon identified the lumen-negative transepithelial voltage (Vt) in the ATL under free-flow conditions, suggesting that Na^+ was actively reabsorbed in this segment [6]. Later *in vivo* studies using electrodes with increased reliability found a lumen-positive Vt under free-flow conditions in the ATL [3–7], but did not resolve the issue of whether active Cl^- reabsorption was responsible for lumen-positive potentials. *In vitro* studies demonstrated the absence of active NaCl reabsorption in the ATL and found no evidence for a spontaneous Vt or net reabsorption of NaCl [8–10]. As for Cl^- transport in the ATL, several studies have provided substantial evidence to support the presence of a transcellular conductive Cl^- transport system apparently consisting of Cl^- channels in both the luminal and basolateral membranes in the ATL [11–14]. A recent study using molecular cloning techniques revealed the presence of a Cl^- channel that is highly specific for the ATL, supporting the view that Cl^- is passively transported across ATL cells [15]. As for the nature of Na^+ reabsorption in the ATL, Koyama, Yoshitomi and Imai demonstrated the presence of protamine-sensitive Na^+ permeability, indicating that the tight junction of the ATL is highly permeable to Na^+ [16]. However, they showed that only 30% of transmural Na^+ permeability is dependent on paracellular pathway [16]. Therefore, their study cannot rule out the possibility that the rest of the Na^+ permeability might be due to the transcellular pathway. We recently demonstrated that the hamster ATL possesses ouabain-sensitive Na^+/K^+ATPase in the basolateral membrane which maintains a low intracellular sodium concentration ($[Na^+]_i$) [17]. Although the presence of a potent Na^+ pump implies the presence of active Na^+ reabsorption, our mathematical estimation of the net transport rate of Na^+ across ATL cells clearly indicates that ATL cell membranes are not organized actively to reabsorb a physiologically significant amount of Na^+ across the cells. We concluded that most of the Na^+ reabsorption in the ATL does not occur transcellularly. However, the previous works did not exclude the possibility that several percentages of Na^+ reabsorption might be attributed to the transcellular pathway (active Na^+ reabsorption), because we observed a change in $[Na^+]_i$ in response to a change in luminal Na^+ concentration in the absence of peritubular Na^+ [17]. But, properties of Na^+ transport across the cell membranes of the ATL other than Na^+/K^+ATPase in the basolateral membrane were not characterized then.

In our recent study using a pH-sensitive fluorescent probe, BCECF, we found that a Na^+/H^+ antiporter is present only in the basolateral membrane of the ATL and that this transporter is the

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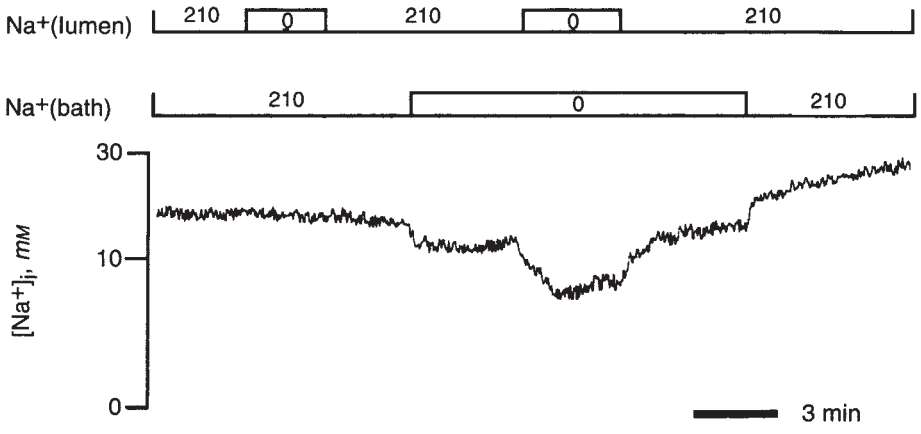

Fig. 1. *Representative tracing showing the effect of removal and replacement of luminal Na^+ on $[Na^+]_i$ in the ATL in the presence and absence of bath Na^+.* First, the tubule was bathed in and perfused with solution A. The luminal solution was replaced with solution B to eliminate Na^+. After replacement of luminal Na^+, peritubular Na^+ was removed by replacing solution A with solution B. Then luminal Na^+ was again removed and replaced. Changes in luminal Na^+ concentration did not change $[Na^+]_i$ in the presence of bath Na^+. In contrast, $[Na^+]_i$ changed upon changes in luminal Na^+ concentration in the absence of bath Na^+

Table 1. Composition of artificial solutions

	A	B	C	D	E	F
Na^+ mM	210.0	—	210.0	210.0	210.0	—
$NMDG^+$ mM	—	210.0	—	—	—	185.0
$Choline^+$ mM	—	—	—	—	—	25.0
Cl^- mM	208.0	208.0	208.0	193.0	215.5	193.0
Hepes mM	10.0	10.0	10.0	—	—	—
HCO_3^- mM	—	—	—	25.0	2.5	25.0
pH	7.4	7.4	6.4	7.4	6.4	7.4

All solutions contain the following (in mM): 5.0 K^+, 1.5 Ca^{2+}, 1.0 Mg^{2+}, 2.0 $H_2PO_4^-$, 5.5 glucose, 5.0 l-alanine. Abbreviation is NMDG, N-methyl-D(-)-glucamine. Solutions A to C were equilibrated with 100% O_2. Solutions D to F were equilibrated with 5% CO_2-95% O_2.

main regulator of intracellular pH [18]. Because Cl^- transport in the ATL is sensitive to changes in intracellular pH [12], changes in intracellular Na^+ activity may regulate transcellular Cl^- reabsorption. An understanding of the properties of Na^+ transport across the cell membrane is critical for the elucidation of the regulation of Cl^- reabsorption, which is now considered to be one of the most important roles of the ATL in concentrating urine.

The present study was therefore undertaken to characterize the undefined component of Na^+ transport across the cell membranes of the ATL. The results indicate that Na^+ transport across the cell membrane of the ATL is conducted by the Na^+/H^+ antiporter in the basolateral membrane together with Na^+/K^+ATPase in the basolateral membrane, and demonstrate the absence of luminal Na^+ permeability, that is, the absence of transcellular Na^+ reabsorption. Our results support the passive theory of the countercurrent multiplier model for concentrating urine in the inner medulla of the kidney.

Methods

In vitro microperfusion of isolated tubules

The ATL was microdissected and microperfused *in vitro* on an inverted microscope as previously described [17]. Briefly, male golden hamsters weighing 50 to 80 g were anesthetized with an intraperitoneal injection of 50 mg/kg of pentobarbital sodium and the left kidneys were removed. A fragment of the ATL was microdissected under a stereoscopic microscope with fine forceps in a chilled dish containing N-[2-hydroxyethyl]piperazine-N'-[2-ethanesulfonic acid] (Hepes)-buffered solution A (Table 1). The ATL was then transferred to a perfusion chamber (2 mm in width) mounted on the stage of an inverted microscope (IMT-2, Olympus Co. Ltd., Tokyo, Japan). The distal end of the ATL with a fragment of the medullary thick ascending limb was sucked into a glass pipette, and the lumen of the ATL was cannulated. The ATL was microperfused with solution A preheated at 37°C and equilibrated with 100% O_2. The bathing solution was continuously exchanged at a rate of 5 ml/min to achieve complete exchange of the solution within one second.

Measurement of intracellular Na^+ activity

$[Na^+]_i$ was monitored using sodium-binding benzofuran isophthalate (SBFI), a fluorescent probe, as previously described [17, 19]. Briefly, SBFI in the form of acetoxymethyl ester (SBFI/AM) was loaded into the ATL cells via the tubular lumen. A 1 μl aliquot of stock solution of SBFI/AM 40 mM in DMSO was mixed with the same amount of 25% Pluronic F-127 in DMSO and diluted with 2 ml of solution A. This solution was then injected into the lumen of the ATL by exchanging the luminal solution in the perfusion pipette. After the ATL was microperfused in the perfusion chamber at 37°C for approximately one hour, the SBFI/AM in the lumen of the ATL was washed out thoroughly with solution A. Fluorescence was measured on an inverted microscope equipped with the OSP-3 system (Olympus Co. Ltd., Tokyo, Japan). The dye trapped in the cells was excited alternately at 340 and 380 nm and the ratio of the intensities of light emitted at 510 nm was converted to Na^+ concentration after the background fluorescence was subtracted. Intracellular SBFI was calibrated at the end of each experiment using amphotericin B as previously described [17].

Solutions

The composition of the solutions used in this study are listed in Table 1.

Chemicals

SBFI/AM was purchased from Molecular Probes, Inc. (Eugene, OR, USA). 5-(N,N-dimethyl)-amiloride HCl was purchased from Research Biochemicals, Inc. (Natick, MA, USA). Hepes, amiloride and furosemide were obtained from Sigma Chemical Company (St. Louis, MO, USA). Trichlormethiazide was a gift from Shionogi Pharmaceutical Company (Osaka, Japan). All other

Table 2. Effects of inhibitors, pH, and HCO_3^- concentration on $[Na^+]_i$ in the ATL

	DMA	Benz	FUR	TCM	pH 6.4	Low HCO_3^-
Lumen						
C	12.6 ± 1.5	11.2 ± 2.1	12.3 ± 1.4	11.5 ± 2.2	10.5 ± 1.5	10.9 ± 1.4[a]
E	12.6 ± 0.9	10.8 ± 1.7	12.4 ± 1.5	11.5 ± 2.2	10.0 ± 1.5	10.6 ± 1.4[a]
Bath						
C	20.2 ± 3.6	20.0 ± 2.1	17.9 ± 2.1	18.7 ± 2.2	16.0 ± 2.4	17.4 ± 1.8[b]
E	9.6 ± 1.5[c]	21.2 ± 2.7	18.4 ± 2.1	19.5 ± 2.0	14.0 ± 2.2	17.4 ± 1.8[b]

The effects of inhibitors, pH, and HCO_3^- concentration in the luminal solution in the absence of peritubular Na^+ are shown under Lumen, C and E. Tubules were bathed in solution B and perfused with solution A. Abbreviations are: DMA, 0.1 mM 5-(N,N-dimethyl)-amiloride; Benz, 1 μM benzamil; FUR, 0.1 mM furosemide; TCM, 0.1 mM trichlormethiazide. C, control; E, experimental period. An inhibitor was added to the lumen during the experimental period. Perfusate was replaced with solution C to acidify luminal pH to 6.4 during the experimental period.

[a] Tubules were bathed in solution F and perfused with solution D. Perfusate was replaced with solution E to decrease luminal HCO_3^- concentration during the experimental period. Results are mean ± SE of 6 tubules. Data are shown in mM.

The effects of inhibitors, pH, and HCO_3^- concentration in the peritubular solution are shown under Bath, C and E. Tubules were bathed in and perfused with solution A. An inhibitor was added to the peritubular solution during the experimental period. Bathing solution was replaced with solution C to acidify peritubular pH to 6.4 during the experimental period.

[b] Tubules were bathed in and perfused with solution D. Bathing solution was replaced with solution E to decrease bath HCO_3^- concentration during the experimental period. Results are mean ± SE of 6 tubules (DMA, $N = 5$)

[c] $P < 0.05$ compared with control

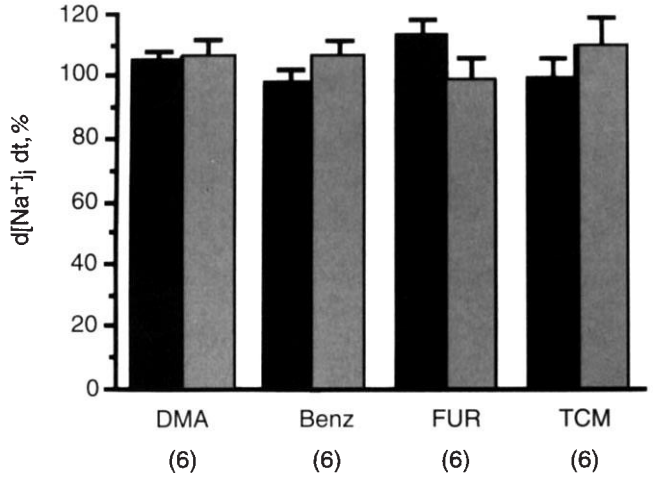

Fig. 2. *Effects of Na^+ transport inhibitors added to the lumen on changes in $[Na^+]_i$ upon removal and replacement of luminal Na^+ in the absence of peritubular Na^+.* Tubules were bathed in solution B and perfused with solution A. After removal (solution B) and replacement of luminal Na^+, 5-(N,N-dimethyl)-amiloride (DMA) in 0.1 mM, benzamil (Benz) in 1 μM, furosemide (FUR) in 0.1 mM, or trichlormethiazide (TCM) in 0.1 mM was added to the luminal solution. After more than 5 min, luminal Na^+ was again removed and replaced by using solutions B and A with an inhibitor. The rates of change in $[Na^+]_i$ ($d[Na^+]_i/dt$) after removal (closed bar) and replacement (hatched bar) of luminal Na^+ in the presence of inhibitors are shown as % of those without inhibitors. The number of tubules is shown in parentheses.

chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Statistical analysis

The data were analyzed using the paired *t*-test. Results are expressed as mean ± SE. A *P* value of <0.05 was accepted as statistically significant. The rate of change in $[Na^+]_i$ ($d[Na^+]_i/dt$, in mM/min) was calculated from changes in $[Na^+]_i$ in the first 30 seconds after the solution was changed.

Results

Na^+ transport in the luminal membrane

Basal $[Na^+]_i$ in the ATL was 19.0 ± 1.2 mM ($N = 24$). Removal and replacement of luminal Na^+ did not change $[Na^+]_i$ in 6 tubules. In the absence of peritubular Na^+, removal of luminal Na^+ decreased $[Na^+]_i$ from 11.6 ± 0.9 mM to 6.3 ± 0.8 mM ($N = 21$, $P < 0.0005$). Replacement of luminal Na^+ increased $[Na^+]_i$ to 12.6 ± 0.9 mM ($P < 0.0005$). A representative trace is shown in Figure 1.

The following studies examining the properties of luminal Na^+ transport were conducted in the absence of Na^+ in the bath. The addition of 0.1 mM 5-(N,N-dimethyl)-amiloride (DMA), 1 μM benzamil, 0.1 mM furosemide or 0.1 mM trichlormethiazide to the luminal solution did not change basal $[Na^+]_i$ (Table 2). In the presence of luminal DMA, benzamil, furosemide or trichlormethiazide, the rates of change in $[Na^+]_i$ after removal and replacement of luminal Na^+ did not differ from that of the control (Fig. 2). Ten μM amiloride also did not affect the luminal Na^+ transport (data not shown). There was no significant change in $[Na^+]_i$ associated with decreases in luminal pH in a Hepes-buffered solution and luminal HCO_3^- concentration (Table 2). In contrast, DMA in the bathing fluid significantly reduced $[Na^+]_i$ from 11.4 ± 1.3 to 6.4 ± 1.2 mM ($P < 0.01$, $N = 5$) in the absence of Na^+ in the bath (Fig. 3A). Changes in $[Na^+]_i$ after changes in luminal Na^+ concentration were completely inhibited by DMA in the bath, to values that were not significantly different from zero (Fig. 3 A, B).

Na^+ transport in the basolateral membrane

When basolateral Na^+ was removed, $[Na^+]_i$ decreased from 18.8 ± 1.2 mM to 11.3 ± 0.7 mM ($P < 0.0001$, $N = 23$). When Na^+ was added to the basolateral side of the tubules, $[Na^+]_i$ recovered to 20.4 ± 1.3 mM. Addition of 10 μM DMA to the bath tended to reduce basal $[Na^+]_i$ and $d[Na^+]_i/dt$, but these changes were not significant. Addition of 0.1 mM DMA to the bath reduced $[Na^+]_i$ (Table 2) and inhibited the changes in $[Na^+]_i$ after removal and replacement of bath Na^+ (Fig. 4). But the addition of 1 μM benzamil, 0.1 mM furosemide or 0.1 mM trichlormethiazide to the

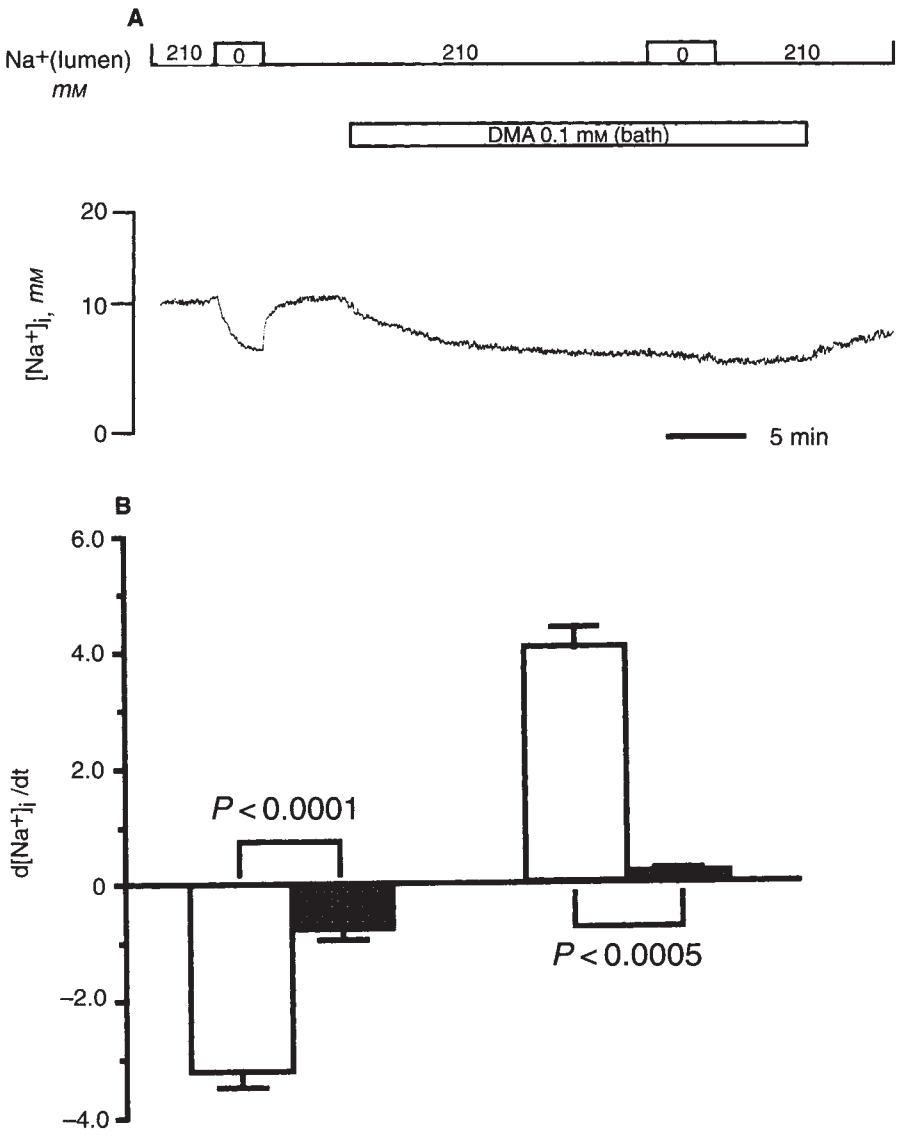

Fig. 3. *Effect of DMA in the peritubular solution on $[Na^+]_i$ and changes in $[Na^+]_i$ upon removal and replacement of luminal Na^+ in the absence of peritubular Na^+.* **A.** Representative tracing. **B.** Effect of peritubular DMA on $d[Na^+]_i/dt$ after removal and replacement of luminal Na^+. Open bar, control; hatched bar, in the presence of peritubular DMA (N = 5). First, tubules were bathed in solution B and perfused with solution A. After removal (solution B) and replacement of luminal Na^+, DMA in 0.1 mM was added to the peritubular solution. Then, luminal Na^+ was again removed and replaced.

bath did not alter $[Na^+]_i$ or the rate of change in $[Na^+]_i$ after removal and replacement of bath Na^+ (Table 2, Fig. 4). There was no significant change in $[Na^+]_i$ when either basolateral pH or basolateral HCO_3^- concentration was decreased (Table 2). These results indicate that the basolateral membrane of the ATL possesses a DMA-sensitive Na^+ transport system but it does not have a functional Na^+ channel, Na^+-K^+-$2Cl^-$ cotransporter, Na^+-Cl^- cotransporter, or HCO_3^--coupled Na^+ transporters.

Discussion

The ATL plays an important role in the formation of concentrated urine by the countercurrent multiplier system. We have recently identified an ouabain-sensitive Na^+/K^+ATPase found exclusively in the basolateral membrane, which maintains a low $[Na^+]_i$ in the hamster ATL [17]. The present study was designed to investigate further the Na^+ transport processes in the luminal and basolateral membranes in this segment.

Na^+ transport mechanism of the luminal membrane

In our previous study, we observed a change in $[Na^+]_i$ in response to a change in luminal Na^+ concentration in the absence of peritubular Na^+ [17]. But, as shown in the present study (Fig. 1), $[Na^+]_i$ was not modified by changes in luminal Na^+ concentration in the presence of peritubular Na^+. The previous observation was consistent with but did not prove the presence of apical Na^+ permeability. In order to clarify the nature of the apparent luminal Na^+ transport, we examined the effects of several transport inhibitors. Neither amiloride, benzamil, DMA, furosemide nor trichlormethiazide in the lumen affected $[Na^+]_i$ or the rate of change in $[Na^+]_i$ ($d[Na^+]_i/dt$) after removal and replacement of luminal Na^+, indicating that the luminal membrane of the ATL does not possess a Na^+ channel, Na^+/H^+ antiporter, or Na^+-K^+-$2Cl^-$ or Na^+-Cl^- cotransporters. The absence of a Na^+/H^+ antiporter in the luminal membrane is compatible with our recent

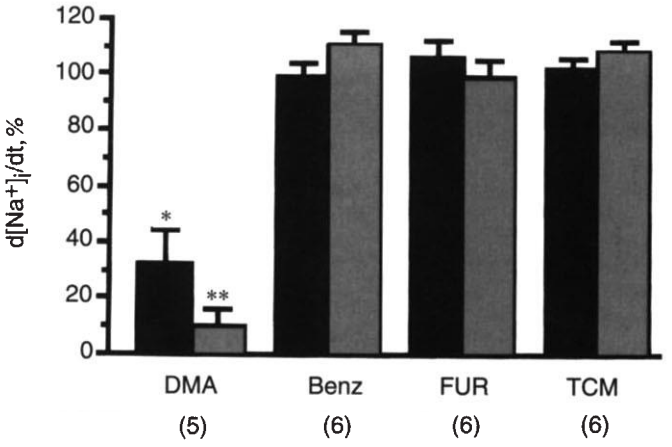

Fig. 4. *Effects of Na^+ transport inhibitors added to the bath on changes in $[Na^+]_i$ upon removal and replacement of peritubular Na^+.* Tubules were bathed in and perfused with solution A. After removal (solution B) and replacement of basolateral Na^+, DMA in 0.1 mM, Benz in 1 μM, FUR in 0.1 mM, or TCM in 0.1 mM was added to the bathing solution. After more than 5 min, peritubular Na^+ was again removed and replaced by using solutions B and A with an inhibitor. $d[Na^+]_i/dt$ after removal (closed bar) and replacement (hatched bar) of peritubular Na^+ in the presence of inhibitors are shown as % of those without inhibitors. $^*P < 0.005$, $^{**}P < 0.0005$.

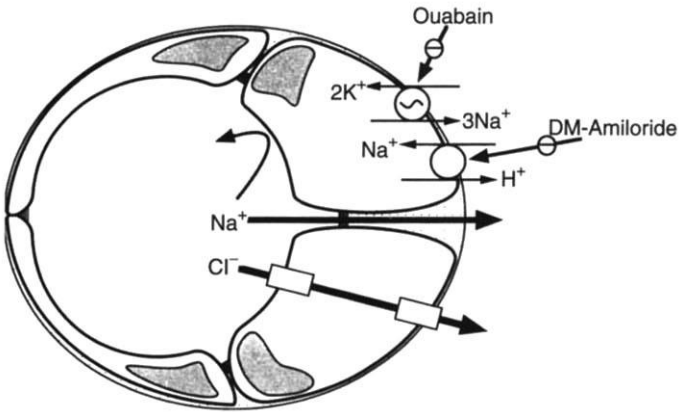

Fig. 5. *Schematic representation of a proposed model of ion transport pathways across the luminal and basolateral membranes and the tight junction in hamster ATL.* Na^+ reabsorption occurs through a paracellular shunt pathway. Cl^- reabsorption occurs through a transcellular route mediated by the Cl^- channel which is inhibited by 4,4′-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS), 4-acetamido-4′-diisothiocyanostilbene (SITS), 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB) and phloretin [11]. Only the Cl^- channel in the basolateral membrane is sensitive to furosemide [11]. The luminal membrane of the ATL lacks Na^+ permeability and the basolateral membrane of the ATL possesses ouabain-sensitive Na^+/K^+-ATPase [17] and a DMA-sensitive Na^+/H^+ antiporter, which are the main regulators of $[Na^+]_i$.

data [18]. We examined the effect of removal of luminal Cl^- on $[Na^+]_i$ and found that Cl^- transport was not coupled with Na^+ transport in the luminal membrane (data not shown). HCO_3^--coupled Na^+ transporters are also absent in the luminal membrane, which is compatible with our recent data [18]. Our preliminary data using fura-2 showed the absence of a Na^+/Ca^{2+} antiporter in the luminal membrane of the ATL (data not shown).

Because we were not able to obtain positive proof of the presence of apical Na^+ permeability, we examined whether a change in $[Na^+]_i$ after a change in luminal Na^+ concentration reflected basolateral Na^+ permeability. It is possible that the Na^+ involved in this process came from the lumen across the paracellular pathway. Surprisingly, DMA in the bath reduced $[Na^+]_i$ in the absence of peritubular Na^+ and completely inhibited the changes in $[Na^+]_i$ after changes in luminal Na^+ concentration. This result may be explained as follows. In the absence of Na^+ in the bath, luminal Na^+ continuously flows out to the basolateral side through the tight junction. Leaked Na^+ then enters the cells through basolateral DMA-sensitive Na^+ transporters and increases the basal $[Na^+]_i$ under these experimental conditions. Because the permeability of the tight junction is extraordinarily high, Na^+ concentration in the basolateral microenvironment beside the cells is partially equilibrated with that in the lumen. Once the basolateral Na^+ transporters are blocked, Na^+ in the lumen is unable to flow into the cells across the basolateral membrane of the ATL. Then basal $[Na^+]_i$ decreases and changes in luminal Na^+ concentration no longer alter $[Na^+]_i$. It is unlikely that DMA inhibits the apical Na^+ transport pathway only from the cytoplasmic side and that DMA enters the cells only across the basolateral membranes, considering the following four observations: (1) amiloride exerts its inhibitory effect at the external aspect of Na^+/H^+ antiporters [20–23]; (2) deletion of the cytoplasmic domain of the Na^+/H^+ antiporter preserves amiloride-sensitive Na^+/H^+ exchange [24]; (3) the amiloride binding site is in the fourth putative transmembrane segment of the Na^+/H^+ antiporter protein [25]; and (4) 1 mM DMA in the lumen did not affect Na^+ transport in the ATL (data not shown). In our preliminary study, we tested the effect of protamine, a tight junction inhibitor, on changes in $[Na^+]_i$ after changes in luminal Na^+ concentration in the absence of bath Na^+. We did not observe any effect of protamine. Koyama, Yoshitomi and Imai demonstrated by a transmural $^{22}Na^+$ flux study that protamine inhibits 30% of the tight junction permeability [16]. Because it is very difficult to detect only 30% of the changes in paracellular flux by monitoring $[Na^+]_i$, protamine is not a useful tool to demonstrate directly the role of the paracellular shunt in changes in $[Na^+]_i$ after changes in luminal Na^+ concentration. Our hypothesis must be proved by complete inhibition of the tight junction by specific substances, which are not available currently. Our present results, however, suggest the absence of luminal Na^+ permeability in the ATL and demonstrate that a change in $[Na^+]_i$ after a change in luminal Na^+ concentration in the absence of bath Na^+ does not indicate the presence of luminal Na^+ permeability. Rather, this reflects Na^+ movement from the lumen to the paracellular pathway, followed by uptake across the basolateral membrane. Therefore, Na^+ reabsorption occurs entirely across the paracellular shunt pathway.

Na^+ transport mechanism of the basolateral membrane

First, we examined the effects of several Na^+ transport inhibitors on Na^+ transport across the basolateral membrane. We were not able to add amiloride to the peritubular solution because of its fluorescent property. In contrast, DMA in the bathing solution at concentrations up to 0.1 mM did not show fluorescence. In the present study, DMA blocked Na^+ permeability in the basolateral membrane by more than 90%, indicating that a DMA-sensitive Na^+ transporter plays an important role in Na^+ transport across

the cell membrane. The present studies also indicate that a Na^+ channel, NaCl cotransporter, NaK2Cl cotransporter, or HCO_3^--coupled Na^+ transporters are not present in the basolateral membrane of the ATL, implying that these transporters do not play significant roles in Na^+ transport across the cell membrane. Our preliminary data using fura-2 showed the absence of a Na^+/Ca^{2+} antiporter in the basolateral membrane of the ATL (data not shown). Kondo, Yoshitomi and Imai demonstrated in the *in vitro* microperfused hamster ATL that furosemide blocks Cl^- transport exclusively from the basolateral side and that over 95% of the Cl^- transport in the ATL is not coupled with Na^+ [11]. Our present data showing that furosemide did not inhibit Na^+ permeability in the basolateral membrane support the view that furosemide inhibits the Cl^- channel in the basolateral membrane. It is not clear why furosemide inhibits the Cl^- channel only in the basolateral membrane. Further studies are required to clarify this point. Together with our recent data, which identified a Na^+/H^+ antiporter in the basolateral membrane of the ATL as the main regulator of intracellular pH [18], the present study indicates that the Na^+/H^+ antiporter in the basolateral membrane is not only the main regulator of intracellular pH but also is the main regulator of $[Na^+]_i$.

Postulated transport model of NaCl in the ATL

Figure 5 provides a schematic representation of Na^+ and Cl^- transport mechanisms in the hamster ascending thin limb of Henle's loop based on our findings. Major components of Cl^- reabsorption occur through a transcellular route. All the Na^+ is reabsorbed across the paracellular pathway. An amiloride-sensitive Na^+/H^+ antiporter and ouabain-sensitive Na^+/K^+ATPase are present in the basolateral membrane, and these are the main $[Na^+]_i$ regulators.

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Reprint requests to N. Takahashi, M.D., Department of Clinical Biology and Hormonal Regulation, Tohoku University School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai 980-77, Japan.

Appendix. Abbreviations

ATL, ascending thin limb; $[Na^+]_i$, intracellular sodium concentration; SBFI, sodium-binding benzofuran isophthalate; DMA, 5-(N,N-dimethyl)-amiloride; BCECF, 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein; DMSO, dimethyl sulfoxide; Benz, benzamil; FUR, furosemide; TCM, trichlormethiazide; $d[Na^+]_i/dt$, rate of change in $[Na^+]_i$.

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